



Food & Beverage Series

SIMULTANEOUS MEASUREMENT OF GLUCOSE
AND SUCROSE UTILIZING EXTERNAL HYDROLYSIS

Application Note 204LS
YSI Life Sciences



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Simultaneous Measurement of Glucose and Sucrose Utilizing External Hydrolysis



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INTRODUCTION

Dextrose (D-glucose) and sucrose concentrations in complex matrices can be measured directly and quickly using the YSI 2900 Series Biochemistry Analyzer. YSI's unique enzyme technology provides for rapid glucose and sucrose measurement. Measurements are virtually unaffected by color, turbidity, density, pH, or the presence of reducing substances.

When a YSI 2900 Series Biochemistry Analyzer is equipped with a glucose membrane, both sucrose and glucose concentrations can be measured. This is accomplished by first determining the glucose concentration. The sucrose is then converted to glucose, and the total glucose concentration is measured. The difference in the responses corresponds to the sucrose concentration.

After a sample is injected into the sample chamber, the glucose diffuses to the glucose membrane, which contains glucose oxidase, and is oxidized to hydrogen peroxide and D-glucono- δ -lactone. The hydrogen peroxide is detected amperometrically at the platinum electrode surface. The current produced is directly proportional to the hydrogen peroxide and glucose concentrations.

For more information on this system, refer to the Operations Manual.

I. MATERIALS & SETUP

- A. YSI 2900 Series Biochemistry Analyzer - equipped with a 2365 Glucose Membrane and 2357 Buffer.
- B. Glucose (1.80 g/L) standard solution.
- C. Buffer Diluent (40 g/L NaH_2PO_4 , 10 g/L Na_2HPO_4 in reagent water).
- D. Invertase - Sigma Chemical Company I-4504 recommended.
- E. Connect the 2900 Series instrument to a suitable power source.

- F. Perform the instrument and membrane check described in the Operations Manual.
- G. Volumetric glassware (Class A recommended).
- H. The following instrument setup is recommended:
Sample Size 25 μL

Probe A Parameters

Chemistry	Glucose
Unit	g/L
Calibrator	1.80 g/L
End Point	30 Sec

Autocal Parameters

Temperature	1°C
Time	30 Min
Sample	5 Sam
Cal Shift	2%

II. METHOD

In this section two different case studies will be described. These examples can be followed when doing analysis on products that may contain similar concentrations of glucose and sucrose.

Case #1

Example: The product is a powdered seasoning mix that is believed to contain 14% glucose and 2% sucrose. The following sample preparation was used:

- A. Weigh out about 10 grams of the powder (record exact weight).
- B. Transfer the powder to a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about 20 minutes.
- C. Remove about 3 mL of the solution in B and add ~2 mg of invertase enzyme. Stir gently until dissolved. Cover the sample and allow to incubate at room temperature for 20 minutes. *continued*

- D. Calibrate the 2900 Series with 1.80 g/L glucose standard solution.
- E. Check the linearity of the membrane at least once a day by injection of an appropriate linearity standard. Refer to the Operations Manual for specifications.
- F. Assay the sample prepared in B by aspiration into the 2900 Series instrument. This is the free glucose concentration (D_{free}).
- G. Assay the sample prepared in C (with invertase). The value reported is the sum of the free glucose and that produced from sucrose hydrolysis (D_{total}).
- H. Calibrate frequently as described in the Operations Manual.

Case #2

The product is a hard candy that is believed to contain 13% sucrose and 12% glucose. With this sample two separate dilutions are necessary. This is due to the glucose membrane reading both free glucose and the glucose produced from the hydrolysis of sucrose. The sum of these two concentrations exceeds the linear range. When analyzing the sample treated with invertase a more dilute sample will be needed. The following sample preparation was used:

- A. Grind the sample into a fine powder.
- B. Transfer 10 grams (record exact weight) of sample, from step A into a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix. Allow the solution to equilibrate for about 20 minutes.*
- C. Transfer 5 grams (record exact weight) of sample, from step A into a 100 mL volumetric flask using buffer diluent to rinse and dilute. Fill the flask to the mark with buffer and mix.
- D. Remove about 3 mL of the solution from C and add ~2 mg of invertase enzyme. Stir gently until dissolved. Cover the sample and allow to incubate at room temperature for about 20 minutes.
- E. Calibrate the 2900 Series with a 1.80 g/L glucose standard solution.
- F. Check the linearity of the membrane at least once a day by injection of an appropriate linearity standard.

Refer to the Operations Manual for specifications.

- G. Assay the sample prepared in B by aspiration into the 2900 Series instrument. This is the free glucose concentration (D_{free}).
- H. Assay the sample prepared in C (with invertase). The value reported is the sum of the free glucose and that produced from sucrose hydrolysis (D_{total}).
- I. Calibrate frequently as described in the Operations Manual.

III. CALCULATIONS

Case #1

To calculate % glucose, multiply the reported value (D_{free}) by the appropriate dilution factor.

Example: A 10.10 g powdered seasoning mix sample was prepared as described in II. B and C. When assayed, the value reported (D_{free}) was 14.6 g/L glucose.

$\begin{aligned} \text{\% Glucose:} \\ 14.6 \text{ g/L} \times 0.100 \\ \text{L}/10.10 \text{ g} \end{aligned}$	$\begin{aligned} &= 0.1446 \text{ gram glucose/gram} \\ &\text{powdered mix} \\ &= 14.5\% \text{ (w/w)} \end{aligned}$
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To calculate % sucrose, subtract D_{free} from D_{total} and multiply by the appropriate dilution and mass ratio factors.

When the sample containing invertase was assayed, the value reported was 15.8 g/L (D_{total}) glucose.

$\begin{aligned} \text{\% Sucrose:} \\ (15.8 \text{ g/L} - 14.6 \text{ g/L}) \times \frac{0.100 \text{ L}}{10.10 \text{ g}} \times \frac{342.30 \text{ g/L sucrose}}{180.16 \text{ g/L glucose}} \end{aligned}$	$\begin{aligned} &= 0.0226 \text{ gram sucrose/gram powdered mix} \\ &= 2.26\% \text{ (w/w)} \end{aligned}$
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Case #2

To calculate % glucose, multiply the reported value (D_{free}) by the appropriate dilution factor.

Example: A 10.10 g ground hard candy sample was prepared as described in II B. When assayed the value reported (D_{free}) was 12.3 g/L glucose.

continued

% Glucose:

$$(12.3 \text{ g/L} \times 0.100 \text{ L}/10.10 \text{ g} = 0.1218 \text{ gram glucose/gram candy} \\ = 12.2\% \text{ (w/w)})$$

To calculate % sucrose, multiply the reported value (D_{total}) by the appropriate dilution factor. Then subtract D_{free} from D_{total} and multiply by the mass ratio factor.

Example: A 5.05 g ground hard candy sample was prepared as described in II D. When assayed the value reported (D_{total}) was 13.5 g/L.

% Sucrose:

$$(13.5 \text{ g/L} \times 0.100 \text{ L}/5.05 \text{ g} = 0.2673 \text{ gram glucose/gram candy} \\ = 26.7\% \\ 26.7\% - 12.2\% = 14.5\%$$

$$14.5\% \times \frac{342.30 \text{ g/mole sucrose}}{180.16 \text{ g/mole glucose}} \\ = 27.5\% \text{ (w/w) sucrose}$$

ORDERING INFORMATION

YSI Part Numbers:

- 2900 Biochemistry Analyzer
- 2365 Glucose Membrane Kit
- 2747 Glucose Standard Solution (1.80g/L)
- 1531 Glucose Standard Solution (9.0g/L)
- 2357 Buffer Kit
- 2363 Potassium Ferrocyanide Test Solution
- 2392 NaCl Solution (for membrane installation)

For further information, please contact:

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YSI Life Sciences develops and manufactures scientific instruments, sensors and systems that serve a variety of scientific and industrial markets worldwide. YSI has a long history in the life sciences and bioanalytical markets, most notably with our introduction of the world's first commercial whole blood glucose analyzer in 1975. Today there are over 10,000 YSI instruments installed around the world, trusted in critical situations to provide the most accurate data in the shortest time.

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